REMARKS

I. Status of the Claims and Amendments

Claims 18, 19, 22, 28, 29, 31, 32, 38, 39, 42, 53-56 and 65-68 are pending in the application. All claims except claim 66 stand rejected under 35 U.S.C. §112, 35 U.S.C. §102 and 35 U.S.C. §103. The specific grounds for rejection, and applicants' response thereto, are set out in detail below.

Please note the amendment to claim 39. Previously, the term "antibody fragment" had been left out of the claim. This term has been reintroduced, though not through amendment as it was already part of the claim by virtue of the amendment filed on August 11, 2003.

II. Interview and Request for Interview

Applicants wish to thank Examiner Helms for the courtesy of a telephonic interview held on May 12, 2005. Applicants concur with and adopt the examiner's characterization of the May 12, 2005 interview, as set forth in the Interview Summary mailed on May 16, 2005. Applicants further request that, should this response not place the application in condition for allowance, a second telephonic interview be granted.

III. Copending Application U.S. Serial No. 10/325,694

Applicants wish to make the examiner aware of a copending divisional application, U.S. Serial No. 10/325,694, being handled by Examiner D. Blanchard. Submitted with this response is a supplemental IDS including all office actions from the '694 application.

IV. Rejections Under 35 U.S.C. §112, First Paragraph

Claims 22, 42 and 68 stand rejected under the first paragraph of §112 as lacking an enabling disclosure. The examiner argues that claim 22, where one CDR comprises a specific sequence, is non-enabled due to the alleged need for undue experimentation to identify other CDRs that can combine with the specified CDR and bind the 17-1A antigen. Once again, applicants traverse.

First, it must be pointed out that claims 22 and 42 specify 1 of 3 CDRs for each chain, and thus specify 2 of 6 total CDRs. However, in order to make it more clear, applicants have amended the claims to recite that each of the VH and VL chains do indeed comprise 3 CDRs. Second, it cannot be overemphasized that methods of the present invention start with a ***functional*** (*i.e.*, 17-1A antigen binding) antibody. Starting with ***functional*** antibodies or fragments thereof, it would require only ***routine*** experimentation to modify two of the three CDRs of a given chain to obtain those which retained 17-1A binding activity, and comprised one fixed (*i.e.*, unchanged) CDR. Indeed, the independent claim from which claims 22 and 42 depend states that the antibody must recognize the 17-1A antigen. Therefore, the fact that claim 22 specifies (as does claim 42) the particular sequence of a given CDR in each of the VH and VL chain, respectively, does not create an enablement hurdle for the skilled artisan – in fact, this claim is by definition more narrow that the admittedly enabled claims from which it depends. Third, claims 22 and 68 have now been amended to recite that only CDR-encoding portions are used in the creation of the CDR region recited in the claim, thereby obviating an additional concern raised by the examiner during the interview. Thus, it is believed that each of the identified concerns have been addressed.

Claims 32 and 56 are rejected for lack of written description under §112, first paragraph, and alleged new matter (35 U.S.C. §132). According to the examiner, applicants have not

identified support for adding of the SEQ ID NOs. More particularly, the examiner argues that while support exists for inclusion of the added sequences in the alternative, it does not exist for their combination. Applicants traverse. Original claim 32, as set forth in the published PCT application, clearly recited the "and/or" language with regard to the two sequences set forth for each of the VH and VL chains. All the amendment provided was to substitute SEQ ID NOs for the two sequences of FIGS. 7 and 8, and the two sequences of FIGS. 6 and 9, respectively. Thus, no new matter was introduced by this amendment. However, a clarifying amendment is now provided so that the current claim tracks original claim 32 more closely. Moreover, the term "and/or" has been modified to read "or", which embodiment is clearly supported in the published PCT (WO 98/46645) at pages 13 and 14, and page 16 (first paragraph).

Reconsideration and withdrawal of this rejection is again respectfully requested.

V. Rejection Under 35 U.S.C. §112, Second Paragraph

Claim 68 is rejected under the second paragraph of §112 as allegedly indefinite. The examiner argues that the claim is indefinite as it is not clear whether framework regions from the specified sequences are included in the CDRs. In amended claim 68, the three CDRs of the VH chain have been defined in that they are encoded by three ***CDR-encoding segments*** within nucleotides 1 to 381 of Seq. ID NO: 143 (corresponding to the FIG. 7 sequence), whereas the VL chain-CDRs have been defined to be encoded by three CDR-encoding segments within nucleotides 1 to 321 of Seq. ID NO:141 (corresponding to the FIG. 6 sequence). The description, for example, in the figure legend of FIGS. 6 and 7 on page 19 of the corresponding WO 98/46645 indicates the exact positions of the CDRs in the VL and VH chain, respectively, of

the claimed antibody or antibody fragment. Since the amendment to claim 68 makes it clear that no framework regions are included in the CDRS, the objection no longer applies.

VI. Rejection Under 35 U.S.C. §103 over Kucherlapati *et al.* in view of Gottlinger

Claims 18, 19, 28-31, 38, 39, 53-55, 65 and 67 remain rejected as obvious over the '584 patent in view of Gottlinger. Applicants once again traverse.

Amended claim 18 no longer refers to the term "derived from." Thus, it has been clarified that the VH chain of the claimed antibody ***is*** a VH chain of unprimed mature human B-lymphocytes. Moreover, as argued previously, the antibodies of the invention are characterized as being only low or not at all immunogenic in humans. This is achieved by the use of ***unprimed mature human B-lymphocytes*** as the source for the VH chain. The use of such cells results in the absence of amino acid exchanges caused by somatic mutations in at least the VH regions, thereby avoiding immune responses in humans. In contrast, the human antibodies generated in transgenic mice as described in the '584 patent are somatically mutated (see, for example, the attached FIG. 12A of the '584 patent, showing a DNA sequence alignment of the somatically mutated heavy chain of anti tetanus toxin monoclonal antibody D5.1.4 and germline VH6), and thus exhibit some immunogenicity of significance in humans.

In addition, applicants provide further evidence that administration to cancer patients of a human anti-17-1A antibody of the invention is non-immunogenic (Declaration 1). Particularly, a fully human IgG1 antibody based on the V-sequences of the present invention was tested in 20 human prostate carcinoma patients in a Phase I clinical trial with repeated dosing regimen. The patients received two infusions of said antibody with a break of 14 days in between. The first cohort consisting of 2 patients received 10 mg/m^2 body surface area per infusion, whereas the

other six cohorts consisting of 3 patients received 20, 40, 64, 102, 164, and 262 mg/m^2 body surface area, respectively. As demonstrated in Appendix 7, administration of the indicated human anti 17-1A antibody did not elicit an immune response against the human anti 17-1A antibody in any of these patients (no antibodies against the human anti 17-1A antibody could be detected in the serum of these patients on day 28, 35, 42 and 70 after the day of the second infusion). As a positive control, the serum of all patients was spiked with 1µg/ml of an antibody binding to the human anti 17-1A antibody. Appendix 7 exemplifies this control for patient 002004 at day 42 (positive control). Accordingly, none of the 20 patients revealed any detectable antibody titer against the human anti 17-1A antibody clearly underlining the lack of immunogenicity in humans of a human antibody of the present invention. Thus, the present invention provides (two) VH sequences of anti-EpCAM binders derived from the repertoire of unprimed mature human B-lymphocytes. As required for V-regions derived from the pool of unprimed mature human B lymphocytes, these sequences show a 100 % identity to the respective human germline sequences.

It should be noted that, in contrast to the data described in Appendix 7 and summarized above, many therapeutic antibodies used in clinic are immunogenic as set forth in the attached publication by Amin & Carter (Appendix 8). According to the authors, the clinical success of first chimeric (part mouse, part human) and then humanized antibodies (part mouse, mostly human) and recently "fully human" (obtained from human gene repertoires in transgenic mice or recombinant phage libraries) has re-enforced the impression that foreignness is the issue and that humans do not raise antibodies to human proteins or antibodies. However, according to the authors this simplistic view is plainly wrong. Table II on page 21 of said Amin & Carter demonstrates that chimeric, humanized and even fully-human antibodies used in therapy elicit

immune responses in treated patients. The authors therefore concluded that the history of therapeutic antibody engineering actually largely teaches us how to be "more human" which, however, is not necessarily to be "less immunogenic." This is underlined by the fact that even fully-human antibodies can show significant immunogenicity rates, as exemplified by the Humira antibody used in therapy of Crohn's disease and rheumatoid arthritis; see Table II of Amin & Carter.

Furthermore, the '584 patent fails to provide any direction to use of unprimed mature human B-lymphocytes in order to forego somatic mutations and thus immunogenicity in the human organism. Thus, a person skilled in the art would not glean from the teachings of the '584 patent application the use of germline VH sequences, *i.e.*, non-mutated sequences. Accordingly, the teachings of the '584 patent do not enable the production of low or non-immunogenic antibodies, nor was there a reasonable expectation of success for the skilled person to obtain such antibodies. This is true simply because the '584 patent is completely silent about an appropriate B cell source, *i.e.*, unprimed mature human B lymphocytes which allow the generation of germline sequences-derived antibodies according to the invention. The above-mentioned defects in the '584 patent are not cured by Göttlinger *et al.*

However, though the examiner seems to acknowledge these shortcomings of the prior art, it is argued that use of the term "derived from" in the claims opens their interpretation to include use of primed, somatically-mutated sequences like those in the '584 patent. Applicants traverse, but in the interest of advancing the prosecution, applicants have amended claim 18 to recite that the VH chain "is a VH sequence of unprimed mature human B-lymphocytes" and the VL chain "is a VL sequence of a naturally occurring human B cell repertoire." Thus, there now can be no

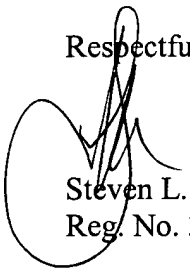
mistaking the fact this antibody is both novel over the cited references, but it also is nowhere suggested.

In sum, the cited art fails to teach each element of the claimed invention - low or non-immunogenic antibodies to 17-1A having V sequences from unprimed B-lymphocytes. As such, the rejection is improper; reconsideration and withdrawal thereof is respectfully requested.

VII. Conclusion

All claims are believed to be in condition for allowance, and an early notification to that effect is earnestly solicited. Should Examiner Helms have any questions regarding this response, a telephone call to the undersigned is invited. Please date stamp and return the enclosed postcard as evidence of receipt.

Respectfully submitted,



Steven L. Highlander
Reg. No. 37,642

Date: June 27, 2005

Fulbright & Jaworski L.L.P.
600 Congress Ave., Suite 2400
Austin TX 78701
512-536-3184

Transmittal Form to Commissioner for Patents
May 12, 2005
Our reference: DEBE:017USD1
Your reference: MIC-001 PCT/US

bcc: Dr. Albrecht Dehmel (w/encl.) for distribution to:
Mr. Peter Kufer
Mr. Tobias Raum

PTO/SB/31 (06-04)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

NOTICE OF APPEAL FROM THE EXAMINER TO THE BOARD OF PATENT APPEALS AND INTERFERENCES

Docket Number (Optional): DEBE:017USD1

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to "Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450" [37 CFR 1.8(a)]
on _____

Signature _____

Typed or printed name Steven L. Highlander

In re Application of: Peter Kufer et al.

Application Number	Filed
10/325,694	December 19, 2002

For NOVEL METHOD FOR THE PRODUCTION OF ANTIHUMAN..

Art Unit	Examiner
1642	D. Blanchard

Applicant hereby **appeals** to the Board of Patent Appeals and Interferences from the last decision of the examiner.

The fee for this Notice of Appeal is (37 CFR 1.17(b)) $ 500.00

☐ Applicant claims small entity status. See 37 CFR 1.27. Therefore, the fee shown above is reduced by half, and the resulting fee is: $ _____

☑ A check in the amount of the fee is enclosed.

☐ Payment by credit card. Form PTO-2038 is attached.

☐ The Director has already been authorized to charge fees in this application to a Deposit Account. I have enclosed a duplicate copy of this sheet.

☑ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. 50-1212/10213812 . I have enclosed a duplicate copy of this sheet.

☑ A petition for an extension of time under 37 CFR 1.136(a) (PTO/SB/22) is enclosed.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

I am the

☐ applicant/inventor.

☐ assignee of record of the entire interest. See 37 CFR 3.71. Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96)

☑ attorney or agent of record. Registration number 37,642

☐ attorney or agent acting under 37 CFR 1.34. Registration number if acting under 37 CFR 1.34. _____

_____
Signature

Steven L. Highlander
Typed or printed name

512-536-3184
Telephone number

_____
Date

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☐ *Total of _____ forms are submitted.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Peter Kufer
Tobias Raum

Serial No.: 10/325,694

Filed: December 19, 2002

For: NOVEL METHOD FOR THE PRODUCTION OF ANTIHUMAN ANTIGEN RECEPTORS AND USES THEREOF

Group Art Unit: 1642

Examiner: D. Blanchard

Atty. Dkt. No.: DEBE:017USD1

CERTIFICATE OF MAILING
37 C.F.R 1.8

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May 12, 2005
Date

Steven L. Highlander

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

MS AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Sir:

In compliance with the duty of disclosure under 37 C.F.R. § 1.56, it is respectfully requested that this Supplemental Information Disclosure Statement be entered and the documents listed on attached Form PTO-1449 be considered by the Examiner and made of record. Copies of the listed documents required by 37 C.F.R. § 1.98(a)(2) are enclosed for the convenience of the Examiner.

In accordance with 37 C.F.R §§ 1.97(g), (h), this Supplemental Information Disclosure Statement is not to be construed as a representation that a search has been made, and is not to be

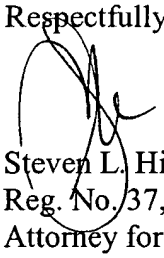
construed to be an admission that the information cited is, or is considered to be, material to patentability as defined in 37 C.F.R. § 1.56(b).

No fees are believed to be due in connection with the filing of this Supplemental Information Disclosure Statement, however, should any fees under 37 C.F.R. §§ 1.16 to 1.21 be deemed necessary for any reason relating to these materials, the Commissioner is authorized to deduct the appropriate fees from Fulbright & Jaworski Deposit Account No.: 50-1212/DEBE:017USD1.

This application is a divisional application of Serial No. 09/403,107, filed October 14, 1999 and is relied upon for an earlier filing date under 35 U.S.C. § 120.

Applicants respectfully request that the listed documents be placed in the file in the present case.

Respectfully submitted,



Steven L. Highlander
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Attorney for Applicants

FULBRIGHT & JAWORSKI L.L.P.
600 Congress Avenue, Suite 2400
Austin, Texas 78701
(512) 474-5201

Date: May 12, 2005

Form PTO-1449 (modified)	Atty. Docket No. DEBE:017USD1	Serial No. 10/325,694
List of Patents and Publications for Applicant's INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)	Applicant Peter Kufer Tobias Raum	
	Filing Date: December 19, 2002	Group: 1642

U.S. Patent Documents	Foreign Patent Documents	Other Art
See Page 1	*See Page 1*	*See Page 1*

U.S. Patent Documents

Exam. Init.	Ref. Des.	Document Number	Date	Name	Class	Sub Class	Filing Date of App.
	A2	6,150,584	11/21/00	Kucherlapati *et al.*	800	18	10/02/96

Foreign Patent Documents

Exam. Init.	Ref. Des.	Document Number	Date	Country	Class	Sub Class	Translation Yes/No

Other Art (Including Author, Title, Date Pertinent Pages, Etc.)

Exam. Init.	Ref. Des.	Citation
	C6	Amin and Carter, "Immunogenicity issues with therapeutic protein," *Curr Drug Disc,* Nov: 20-24, 2004
	C7	Dermer, "Another anniversary for the war on cancer," *Bio/Technology,* 12:320, 1994.
	C8	Freshney, Culture of animal cells: a manual of basic technique, Alan R. Liss, Inc., New York p4, 1983.
	C9	Gura, "Systems for identifying new drugs are often faulty," *Science,* 278:1041-1042, 1997.
	C10	Herlyn and Koprowski, "IgG2a monoclonal antibodies inhibit human tumor growth through interaction with effector cells," *Proc Natl Acad Sci USA,* 79:4761-4765, 1982.
	C11	Herlyn *et al.,* "Colorectal carcinoma-specific antigen: detection by means of monoclonal antibodies," *Proc Natl Acad Sci USA,*76(3):1438-1442, 1979.
	C12	Jain, "Barriers to drug delivery in solid tumors," *Sci Am,* 271(1):58-65, 1994.
	C13	Litvinov *et al.,* "Ep-CAM: a human epithelial antigen is a homophilic cell-cell adhesion molecule," *J Cell Biol,* 125(2):437-446, 1994.
	C14	Office Action dated 01/27/05
	C15	Office Action dated 03/13/03
	C16	Office Action dated 03/27/01
	C17	Office Action dated 03/28/02

25534626.1

EXAMINER:	DATE CONSIDERED:

EXAMINER: INITIAL IF REFERENCE CONSIDERED, WHETHER OR NOT CITATION IS IN CONFORMANCE WITH MPEP609; DRAW LINE THROUGH CITATION IF NOT IN CONFORMANCE AND NOT CONSIDERED. INCLUDE COPY OF THIS FORM WITH NEXT COMMUNICATION TO APPLICANT.

Form PTO-1449 (modified)	Atty. Docket No. DEBE:017USD1	Serial No. 10/325,694
List of Patents and Publications for Applicant's INFORMATION DISCLOSURE STATEMENT (Use several sheets if necessary)	Applicant Peter Kufer Tobias Raum	
	Filing Date: December 19, 2002	Group: 1642

U.S. Patent Documents	Foreign Patent Documents	Other Art
See Page 1	*See Page 1*	*See Page 1*

Other Art (Including Author, Title, Date Pertinent Pages, Etc.)

Exam. Init.	Ref. Des.	Citation
	C18	Office Action dated 05/07/04
	C19	Office Action dated 08/27/02
	C20	Office Action dated 10/28/03
	C21	Rudikoff *et al.*, "Single amino acid substitution altering antigen-binding specificity," *Proc Natl Acad Sci USA*, 79:1979-1983, 1982.
	C22	Winter *et al.*, "Making antibodies by phage display technology," *Annu Rev Immunol*, 12:433-455, 1994.

25534626.1

EXAMINER:	DATE CONSIDERED:

EXAMINER: INITIAL IF REFERENCE CONSIDERED, WHETHER OR NOT CITATION IS IN CONFORMANCE WITH MPEP609; DRAW LINE THROUGH CITATION IF NOT IN CONFORMANCE AND NOT CONSIDERED. INCLUDE COPY OF THIS FORM WITH NEXT COMMUNICATION TO APPLICANT.

CERTIFICATE OF MAILING
37 C.F.R. § 1.8

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May 12, 2005
Date

Steven L. Highlander

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Peter KUFER *et al.*

Serial No.: 10/325,694

Filed: December 19, 2002

For: NOVEL METHOD FOR THE PRODUCTION OF ANTIHUMAN ANTIGEN RECEPTORS AND USES THEREOF

Group Art Unit: 1642

Examiner: D. Blanchard

Atty. Dkt. No.: DEBE:017USD1/SLH

AMENDMENT AND REQUEST FOR RECONSIDERATION UNDER 37 C.F.R. §1.116

MS AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This is in response to the office action mailed on November 12, 2004, to which a response is due on May 12, 2005, by virtue of the enclosed Petition for Extension of Time and payment of fees. No other fees are believed due; however, should applicants' check be missing, or any other fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason, the Commissioner is authorized to deduct said fees from Fulbright & Jaworski L.L.P. Account No.: 50-1212/10213812/SLH. Please date stamp and return the enclosed postcard as evidence of receipt.

Amendments to the Claims begin on page 2 of this paper; **Amendment to the Specification** are on page 6; **Remarks** begin on page 7.

AMENDMENTS

Listin of Claims

The following listing of claims replaces all previous listings or version thereof:

1-33. (Canceled)

34. (Currently amended) A method for the production of an antibody or antibody fragment, the method comprising the steps of (i) combining a human VH or VL chain repetoire with a single VL or VH chain, respectively, from a different species; (ii) selecting either a human VH or a human VL chain for binding to a human target antigen together with ~~a VL and VH chain, respectively, of a target antigen specific antibody from a different species~~the VL or VH chain from said different species; (iii) combining the human VH or human VL chain selected in step (ii) with a human VL or VH chain repetoire such that said VL or VH chain from said different species is replaced by a human VL or VH chain, respectively, and ~~[[(ii)]]~~(iv) selecting a combination of human VH and human VL immunoglobulin chains for binding to said human target antigen, wherein at least said human VH chain is from unprimed mature human B-lymphocytes and said human VL chain is from a naturally occurring human B cell repertoire, these chains being expressed from a recombinant vector by *in vitro* display for binding to the human target antigen.

35. (Previously presented) The method according to claim 34, wherein said antibody is an antibody fragment.

36. (Previously presented) The method according to claim 35, wherein said antibody fragment is an Fv fragment.

37. (Currently amended) The method according to claim 34, wherein either said human VH immunoglobuin chain or both said human VH and said human VL immunoglobulin chains are from a human IgD repertoire.

38. (Previously presented) The method according to claim 34, wherein said *in vitro* display is phage display.

39. (Previously presented) The method according to claim 34, wherein said immunoglobulin chains are expressed from one or more different libraries.

40. (Previously presented) The method according to claim 34, wherein said antibody or antibody fragment is an antibody or antibody fragment directed to a human tumor antigen.

41. (Previously presented) The method according to claim 40, wherein said antibody or antibody fragment recognizes the human 17-1A antigen.

42. (Currently amended) The method according to claim 41, wherein said human ~~VH chain~~VH immunoglobulin chain comprises the amino acids encoded by nucleotides 1 to 381 of Seq. ID No: 143 and said human VL immunoglobuin chain comprises the amino acids encoded by nucleotides 1 to 321 of Seq. ID No.: 141.

43. (Previously presented) The method according to claim 34, wherein said selecting steps comprise:

(i) binding a display vehicle expressing an anti-human antibody or antibody fragment recognizing the target human antigen selected from the group consisting of:
   (a) an immobilized human target antigen or a fragment thereof;
   (b) cells expressing the human target antigen or a fragment thereof; and
   (c) a soluble human target antigen or a fragment thereof;

and a second step selected from the group consisting of:

(ii) removing by washing off the display vehicles that are not bound to (a) or (b) and subsequently eluting the display vehicles that are bound to (a) or (b); and

(iii) positively enriching the human target antigen-bound display vehicles from the suspension of cells expressing the human target antigen (b) or from the human target antigen in (c).

44. (Canceled)

45. (Currently amended) The method according to claim 34, wherein said VH or VL chain from a different species ~~of a non-human target antigen specific antibody~~ used in step (i) is a mouse VH or VL chain.

46. (Currently amended) The method according to claim 34, wherein said selection step (iv) comprises either of the following:

(a) testing one distinct VH chain in combination with a variety of different VL chains for binding to said human target antigen; or

(b) testing one distinct VL chain in combination with a variety of different VH chains for binding to said human target antigen.

47. (Previously presented) The method according to claim 34, further comprising the steps of obtaining, after selection, the human VH and VL chains or the corresponding nucleic acids, and fusing said chains or the corresponding nucleic acids to: (a) the same or other VH or VL chains or the corresponding nucleic acids, (b) immunoglobulin constant regions of heavy (CH) or light chains (CL) or parts thereof or the corresponding nucleic acids, or (c) non-immunoglobulin chains or the corresponding nucleic acids, respectively.

48. (Currently amended) The method according to claim 47, wherein said ~~constant region~~CH or CL chains, or parts thereof, are from human IgGl or [[IgG3]]IgG3.

49. (Currently amended) The method according to claim 34, further comprising the steps of ([[i]]a) obtaining, after selection in step (iv), the human VH and VL immunoglobulin

chains and ([[ii]]b) physically linking said chains to non-proteinous pharmaceuticals and/or other biologically active molecules.

50. (Currently amended) The method according to claim 34, wherein at least said human VH ~~or VL chains are~~immunoglobin chain is expressed from nucleic acid sequences that are RT-PCR amplification products of mRNA from unprimed mature human B-lymphocytes.

51. (Currently amended) The method according to claim 43, wherein either the cells expressing the ~~target~~ human target antigen in (b) of step (i) or the human target antigen in step (c) of step (i) are labeled.

52. (Previously presented) The method according to claim 43, wherein the display vehicles comprising the desired antibody or antibody fragment bound to the human target antigen are multiplied by replication and subjected to further rounds of *in vitro* selection as in steps (i) to (iii).

AMENDMENT TO SPECIFICATION

In the Abstract

Please replace the abstract with the following:

Described is a method for the production of an anti-human antigen receptor that is low or not immunogenic in humans comprising the steps of selecting a combination of functionally rearranged VH and VL immunoglobulin chains wherein at least ~~said~~the VH chain is derived from essentially unprimed mature human B-lymphocytes or from anergic human B cells and ~~said~~the VL chain is derived from a naturally occurring human B cell repertoire, ~~said~~the chains being expressed from a recombinant vector and using an ~~in vitro~~*in vitro* display system for binding to a human antigen. Furthermore, receptors that are low or not immunogenic in humans and directed to human antigens are provided, ~~said~~the receptors being obtainable by the method of the invention. ~~Said~~The receptors are preferably antibodies or fragments thereof or immunoconjugates comprising the VH/VL chains of ~~said~~the antibody. In particular, receptors are described directed to human tumor antigens, preferably to the human tumor antigen 17-1A, also known as EpCAM, EGP, or GA 733-2. Finally, kits useful for carrying out the method of the invention and pharmaceutical compositions comprising the aforementioned receptors are provided.

REMARKS

I. Status of the Claims

Claims 34-43 and 45-52 are pending and stand rejected, variously, under 35 U.S.C. §112, second paragraph, 35 U.S.C. §102 and 35 U.S.C. §103. The specific grounds for rejection, and applicants' response thereto, are set out in detail below.

II. Abstract

The examiner has objected to the use of the term "said" in the abstract. An appropriate amendment has been provided.

III. Supplemental IDS

Applicants are providing a supplemental IDS listing the references attached to this response. In addition, applicants are providing copies of Office Actions mailed in copending parent application, U.S. Serial No. 09/403,107, and the examiner in that case (L. Helms) has been apprised on this instant application (and will be provided with copies of actions in this case).

IV. Rejections Under 35 U.S.C. §112, Second Paragraph

A. Maintained Rejections

The examiner states that because claim 34 recites human VH and VL chains as well as VH and VL from different species, it is unclear what is referred to in dependent claims 37, 42 and 50 when VH and VL are mentioned. Each of these dependent claims has been amended to

recite that the VH and VL referred to is the ***human*** VH and VL of claim 34. Reconsideration and withdrawal of the rejection is respectfully requested.

The examiner also questions whether the constant regions referred to in claim 48 are derived from heavy chains. A clarifying amendment is provided. Reconsideration and withdrawal of the rejection is respectfully requested.

B. New Rejections

Claims 34-43 and 45-52 are rejected over the recitation, in claim 34 of steps (i) and (ii), in light of the view that additional steps are required to provide a human VH/VL pair. Applicants have provided a clarifying amendment that is believed to address the examiner's concerns.

Claims 34-43 and 45-52 are rejected over the recitation, in claim 34, of "target antigen-specific antibody." Applicants have dropped the recitation as suggested by the examiner.

Claim 51 is rejected over the term "target human antigen." A clarifying amendment is provided.

Reconsideration and withdrawal of each of the foregoing rejection is requested.

V. Rejection Under 35 U.S.C. §102

Claims 34-36, 38-39, 43, 45-50 and 52 under §102(e) in view of Hoogenboom *et al.* The patent application of Hoogenboom, in column 11, lines 49 to 52, proposes that the repertoire of rearranged V-genes of unimmunised humans may be used for the selection of human antibodies. Moreover, in column 13, lines 15 and 16, it is suggested that the V chains may be selected from an adult lymphocyte population. Presumably in light of these disclosures, the examiner holds the

view that "as rearranged V-genes of unimmunised humans are unprimed, Hoogenboom *et al.* anticipates the claims." Applicants traverse.

The term "unimmunised humans," as used by Hoogenboom, means humans who have not been immunised with an antigen of interest, *e.g.*, TNF-α, in order to select (*in vitro*) for an antibody against said antigen. It is immediately evident that "immunised" in this context means something distinct from "primed," as will be explained further below. An "unimmunised human," though not immunised with the antigen of interest – ***has encountered and constantly encounters many different antigens from pathogenic and non-pathogenic proteins.*** From these events, priming occurs. Memory B-lymphocytes thus derived circulate through the body (*e.g.*, in the blood stream) until they encounter the same or a similar antigen, and thereby form the basis for a more potent and immediate secondary immune response. Apart from memory B-lymphocytes, plasma cells and IgM-positive lymphoblasts can be found among the peripheral blood cells.

In addition to primed B-lymphocytes, there are also unprimed B-lymphocytes circulating in the blood. These B-lymphocytes have not yet been in contact with any antigen; see the attached scheme on B-lymphocyte maturation (Appendix 1). Thus, the repertoire of rearranged V-genes in B-lymphocytes of unimmunised humans, as referred to by Hoogenboom, comprises pools of primed and unprimed B-lymphocytes. However, Hoogenboom clearly fails to teach or suggest the specific Ig-repertoire of the present invention – unprimed mature B-lymphocytes – as an appropriate source for the generation of low or non-immunogenic human anti-self antibodies according to the method of the present invention. The potential suitability of unprimed mature B-lymphocytes was neither recognized nor taught by Hoogenboom.

Indeed, the antibodies actually selected by Hoogenboom were derived from ***primed*** B-lymphocyte repertoires. Thus, having participated in an immune response, the respective V-regions of the antibodies of these primed B-lymphocytes are somatically mutated. For example, FIGS. 6A and 6B of Hoogenboom show selected sequences from the adult repertoire of healthy "unimmunised" donors, *i.e.*, donors who have not been immunised with TNF-α. All of these sequences clearly show the imprint of somatic hypermutation in the VH as well as in the VL chains (because the B-lymphocytes producing the variable region chains were no longer unprimed when they were employed for the experiments described in Hoogenboom).

In contrast, the V regions from the repertoire of unprimed mature B-lymphocytes according to the invention are characterized by the ***absence*** of somatic mutations due to the fact that these B-lymphocytes have not yet been involved in an immune response. This is confirmed, for example, by VH-regions D4.5 (FIG. 7) and D7.2 (FIG. 8) of the present invention which can be clearly assigned to the germline sequences of ***unprimed*** mature human B-lymphocytes (see the attached sequence alignments of Appendices 2 and 3).

In sum, Hoogenboom clearly fails to teach or suggest use of unprimed mature human B-lymphocytes as an appropriate source for the generation of low or non-immunogenic human anti-self antibodies according to the method of the present invention. Thus, since the claimed method refers to a selection step in which a combination of human VH and VL immunoglobulin chains is selected, wherein at least the human VH chain is from unprimed mature human B-lymphocytes, the method is novel in light of Hoogenboom. Reconsideration and withdrawal of the rejection is therefore respectfully requested.

VI. Rejection Under 35 U.S.C. §103

Claims 34-41, 43 and 45-52 stand rejected under §103(a) in view of Griffiths in combination with Figini and Göttlinger. Applicants traverse.

First, as set forth above, the repertoire(s) of rearranged V-genes of B lymphocytes of an unimmunised human, referred to in both Griffiths and Hoogenboom, is distinct from the repertoire of V-genes from unprimed mature human B-lymphocytes referred to in claim 34 of the present application.

Second, Griffiths further fails to address the problem solved by the present invention, *i.e.*, how to obtain human anti-self antibodies which are low or non-immunogenic in humans. Thus, it also fails to provide or suggest a solution therefor. In light of these observations, applicants submit that there was no motivation for the person skilled in the art to seek for applicants' solution to this problem in view of Griffiths. And further, the secondary references fail to address this issue as well.

Moreover, due to ongoing immune responses found in any individual, as well as circulating memory B-lymphocytes derived from past immune responses, a large portion of blood B-lymphocytes have already participated in host defense. The V-regions of such B-lymphocytes have undergone and will undergo somatic hypermutation in the respective V-sequences when compared to germline sequences. This holds also true for the antibodies provided by Griffiths, as evidenced in the corresponding publication by Griffiths' group (*EMBO J.* 12, 725-734 (1993)). This document describes many different anti-self antibodies, including αThy-29, αTNF-E7, αMUC1-1. However, none of these antibody sequences is free of somatic mutations, as falsely claimed in Table II on p. 76 of WO 93/11236 (Griffiths *et al.*, counterpart to the cited U.S. patent). This is demonstrated by a sequence alignment of the corresponding

sequences with germline VH-segments (Tomlinson, *J. Mol. Biol.* 227, 776-798 (1992)); see Appendices 4-6. Consequently, antibodies obtained according to the methods of Griffiths are clearly not derived from the repertoire of unprimed mature human B-lymphocytes. Thus, the suggestion of using the specific subset of B-lymphocytes claimed by applicants – unprimed mature B-lymphocytes – cannot come from the teachings of Griffiths.

As correctly stated by the examiner, Figini used a "chain-shuffling approach" in that a light chain of a mouse antibody has been utilized to select human heavy chains in order to show that this chimeric molecule is still capable of binding an antigen. However, the reference does not teach use of a unprimed mature human B-lymphocyte population. Göttlinger describes the generation of four mouse monoclonal antibodies recognizing the 17-1A antigen, but does not address selection of a unprimed mature human B-lymphocyte population. Thus, the combination of Griffiths with Figini and Göttlinger fails to teach each element of the claimed invention – namely, methods providing for human antibodies having a low immunogenic profile using unprimed mature human B-lymphocytes.

Further, as discussed above, applicants' invention uses a B-lymphocyte repertoire that provides germline sequences. However, the examiner has not pointed to any teaching that would lead a person skilled in the art to the use of germline VH sequences, *i.e.*, non-mutated sequences. Indeed, each of the cited references is completely silent about applicants' B-lymphocyte source, *i.e.*, unprimed mature human B lymphocytes which allow the generation of germline sequence-derived antibodies according to the invention. All of the selected human antibodies described in Griffiths, Hoogenboom and Figini (see p. 74, Table II, p. 75) are somatically mutated and therefore clearly not derived from unprimed mature B lymphocytes.

Moreover, applicants now provide additional evidence that administration to cancer patients of a human anti-17-1A antibody produced according to the method of the invention is non-immunogenic. *See* Declaration of Mathias Locher (attached). Particularly, a fully human IgG1 antibody based on the V-sequences of the present invention was tested in 20 human prostate carcinoma patients in a Phase I clinical trial with repeated dosing regimen. The patients received two infusions of said antibody with a break of 14 days in between. The first cohort consisting of 2 patients received 10 mg/m^2 body surface area per infusion, whereas the other six cohorts consisting of 3 patients received 20, 40, 64, 102, 164, and 262 mg/m^2 body surface area, respectively. As demonstrated in Appendix 7, administration of the indicated human anti 17-1A antibody did not elicit an immune response against the human anti 17-1A antibody in any of these patients (no antibodies against the human anti 17-1A antibody could be detected in the serum of these patients on day 28, 35, 42 and 70 after the day of the second infusion). As a positive control, the serum of all patients was spiked with 1µg/ml of an antibody binding to the human anti 17-1A antibody. Appendix 7 exemplifies this control for patient 002004 at day 42 (positive control). Accordingly, none of the 20 patients revealed any detectable antibody titer against the human anti 17-1A antibody clearly underlining the lack of immunogenicity in humans of a human antibody produced according to the method of the present invention. Thus, the present invention provides (two) VH sequences of anti-EpCAM binders derived from the repertoire of unprimed mature human B-lymphocytes. As required for V-regions derived from the pool of unprimed mature human B lymphocytes, these sequences show a 100 % identity to the respective human germline sequences.

It should be noted that, in contrast to the data described in Appendix 7 and summarized above, many therapeutic antibodies used in clinic are immunogenic as set forth in the attached

publication by Amin & Carter (Appendix 8). According to the authors, the clinical success of first chimeric (part mouse, part human) and then humanized antibodies (part mouse, mostly human) and recently "fully human" (obtained from human gene repertoires in transgenic mice or recombinant phage libraries) has re-enforced the impression that foreignness is the issue and that humans do not raise antibodies to human proteins or antibodies. However, according to the authors this simplistic view is plainly wrong. Table II on page 21 of said Amin & Carter demonstrates that chimeric, humanized and even fully-human antibodies used in therapy elicit immune responses in treated patients. The authors therefore concluded that the history of therapeutic antibody engineering actually largely teaches us how to be "more human" which, however, is not necessarily to be "less immunogenic." This is underlined by the fact that even fully-human antibodies can show significant immunogenicity rates, as exemplified by the Humira antibody used in therapy of Crohn's disease and rheumatoid arthritis; see Table II of Amin & Carter.

In sum, the cited art fails to teach each an essential element of the claimed method of the invention – use of unprimed mature B-lymphocytes as a source for at least the VH chain, which allow for the generation of low or non-immunogenic antibodies (*e.g.*, to 17-1A) – and it thus fails to establish a *prima facie* case of obviousness. As such, the rejection is improper; reconsideration and withdrawal thereof is respectfully requested.

VII. Conclusion

In light of the foregoing, applicants respectfully submit that all claims are in condition for allowance, and an early notification to that effect is earnestly solicited. Should Examiner

Blanchard have any questions regarding this response, a telephone call to the undersigned is invited.

Respectfully submitted,

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APPENDICES 1-8

FEATURE

Immunogenicity issues with therapeutic proteins

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New models are helping to identify immunogenic risk early in the development process, thereby removing the most major stumbling block in the development of protein therapeutics.

Protein drugs are an increasingly important group of medicines. However, it is apparent that immunogenicity remains an issue for this class of drugs, leading to therapeutic failure, or worse, severe and possibly fatal side effects.

At the same time, for many innovative therapeutic strategies employing non-human proteins, immunogenicity remains an absolute barrier to clinical success.

Regulatory agencies are looking to understand the implications of immunogenicity for the development path and are directing sponsors to build-in programs for immunogenicity risk management from an early point. This means there is a need to consider immunogenicity issues from pre-clinical development, through all clinical trial phases and into the post-registration period.

The issue is set to move further to the fore as the anticipated crop of engineered protein therapeutics arising from gene shuffling or in vitro evolution platforms near clinical development. These molecules with their unique sequences, but usually with more than a passing resemblance to host proteins, are almost certain to run the gamut of immunogenicity risk, irrespective of their projected clinical benefits.

How to offset immunogenicity is also on the minds of those companies seeking to bring generic biologics to market. The regulators are well aware that a big component of biologic product comparability will relate to the management of immunogenicity risk and the demonstration that manufacturing and or formulation changes have not altered the immunogenic profile of the product.

An inevitable consequence of the immunogenicity problem is that a number of companies are focusing on strategies for immune risk limitation. It is possible that, by the application of such methods, more protein drug candidates will fulfill their promise as targeted effective drugs for the future.

Product name	Protein	Indication	% Patients with immune response
ReFacto	Factor VIII	Hemophilia A	~ 30%
Intron A	Interferon α	Hepatitis C	7%
Roferon A			25%
Pegasys			9%
Pegintron			1%
Betaseron	Interferon β	Multiple Sclerosis	10-45%
Avonex			
Rebif			
Eprex	Erythropoietin	Anemia	Non immunogenic Some cases of pure red cell aplasia with Eprex
Aranesp			
Epogen			
Procrit			
Leukine	Granulocyte macrophage colony stimulating factor	Oncology	2.3% (neutralizing antibodies)
Neupogen	Granulocyte colony stimulating factor	Oncology	Non immunogenic
Neulasta			
Enbrel	TNF receptor II human Ig Fc fusion	Rheumatoid arthritis	16%
Proleukin	Interleukin-2	Oncology	74%

Table 1. Immunogenicity of common proteins.

Common and damaging

Immune responses to therapeutic proteins are common. This is testament to the sophistication of an immune system that is able to detect something foreign in our bodies, even if that foreigner shares identity with one of our own proteins.

Should there be any doubt that human proteins can be immunogenic in humans, consider the now greater than 200 patients suffering from pure red cell aplasia following administration of certain

Antibody	Indication	Type of antibody	% Patients with immune response
Remicade	Crohn's Disease & RA	Chimeric	10-60
Simulect	Transplant rejection	Chimeric	1.5
Rituxan	Lymphoma	Chimeric	1.1
ReoPro	Cardiac	Chimeric Fab	6
Anti-A33	Cancer	Humanized	72
Anti – B72.3	Cancer	Humanized	75
CAMPATH	Leukemia	Humanized	50
Zenapax	Transplant rejection	Humanized	8.4
Synagis	RSV Infection	Humanized	1.1
Herceptin	Cancer	Humanized	0.1
Xolair	Allergy	Humanized	0
Humira	Crohn's Disease & RA	Fully-human	12

Table 2. Immunogenicity of therapeutic antibodies.

formulations of recombinant erythropoietin (Table 1). Equally, those receiving preparations of recombinant thrombopoietin, a proportion of patients receiving granulocyte macrophage-colony stimulating factor, interferons and even several of the current crop of therapeutic antibodies give the lie to the notion that immunogenicity is not a problem.

The fact is that immune responses to human and human-like proteins are seen, and while these are benign in the majority of instances, there are patients for whom this has not been the case and indeed previously healthy trial volunteers who have been damaged.

Compounding factors

For some proteins at least, it has been convenient to focus on the notion of 'foreignness' to the immune system as a reason for immunogenicity. We are not surprised that bacterial or plant-derived proteins provide essentially a single-use option, as their repeated use, even in cancer patients, can be rendered ineffective by a neutralizing antibody response or worse.

The history of therapeutic antibody development has taught us that it is possible to engineer proteins to lessen the likelihood of an immunogenic response. For the most part, the solution has been to 'engineer by homology', that is to say recast the antibody sequence to be more similar to the sequences of antibody present naturally in the host. The clinical success of first chimeric (part mouse, part human) and then humanized antibodies (part mouse, mostly human) and recently 'fully human' (obtained from human gene repertoires in transgenic mice or recombinant phage libraries) has re-enforced the impression that foreignness is the issue and that humans do not raise antibodies to human proteins.

Of course, from an immunological standpoint this simplistic view is plainly wrong (Table 2). The immunological decision to either ignore or respond to a therapeutic protein is not based on any simple determination of whether the protein has been seen before. Rather, a number of inputs are sifted wherein factors such as product homogeneity, post-translational modification, degree of aggregation and presence of T-cell epitopes will each come into play. The history of therapeutic antibody engineering actually largely teaches us how to be 'more human' which is not necessarily to be 'less immunogenic' and supporting this there are examples of engineered antibodies with significant immunogenicity rates.

The truth therefore is that there is no single cause of an immune response but rather a matrix of factors that converge to result in an immune response in the first instance, and the clinical consequences (if any) of that response thereafter (Table 3). Thus with the example of erythropoietin and the unfortunate episode of pure red cell aplasia, a likely compounding factor appears to be a formulation change. One working hypothesis centers on the idea that pseudo-viroid type particles comprising polysorbate micelles with erythropoietin protein held in a regular array, has

Product-intrinsic factors	Product-extrinsic factors	Patient Factors
• Human or non-human protein? • Human protein with sequence modifications? • T cell epitopes • Aggregates • Glycosylation status • Impurities • Formulation with HSA	• Route of administration (eg iv versus sc) • Chronic or single dosing • Pharmacokinetics • Cellular or soluble target • Endogenous counterpart	• Immunosuppressed • Autoimmune disease • Replacement therapy (eg genetic disease)

Table 3. Factors in immunogenicity.

resulted in evocation of an immunological danger response. In this, an exaggerated T-helper cell activation has lead to stimulation of a powerful high-affinity antibody response and neutralization of endogenous erythropoietin within afflicted patients.

So, in this instance, we see a scenario where a formulation change possibly altered the immunological perception of an administered protein, with factors such as administration route, T-cell epitopes inherent in the protein and the unique rather than redundant biological activity of the endogenous host protein converging to give an unexpected clinical outcome.

T-cell mediation

Although studies in this area are few, there has been a sense that where immunogenicity has occurred, T-cell involvement is rare. However, the fact that high-affinity class-switched antibodies can be detected to all classes of therapeutic protein should be seen as the fingerprint of a T-cell-mediated response. If so, a critical common factor in such a response is the presence of at least one T-cell epitope within the protein sequence.

At Biovation we take the view that T-cell epitopes are critical elements underlying most immunogenicity seen in the clinic. We have built and refined a number of technical approaches for detecting T-cell epitopes in proteins and along with others have shown the presence of T-cell epitopes in several human proteins including erythropoietin.

A T-cell-mediated response to an administered therapeutic protein involves uptake of the protein by antigen presenting cells (APC) and degradation of the protein into peptide fragments (Figure 1). The peptides will compete for interaction with the binding groove of major histocompatibility complex (MHC) class II molecules, and those that prevail become presented on the surface of the APC in conjunction with their MHC molecule. Where the peptide-MHC complex is able to interact with a specific T-cell receptor on a T-cell, this peptide represents a T-cell epitope. Interaction of the T-cell with the T-cell epitope on the APC can lead to T-cell activation. This is characterized by a proliferative burst from the T-cell and subject to particular patterns of co-stimulatory signals on the APC and the local environment, cytokines stimulatory for the antibody-producing B cells are released. In the absence of T-cell activation, B cells do not undergo maturation to produce high affinity antibody to the incoming antigen nor can become primed to become immunological memory cells.

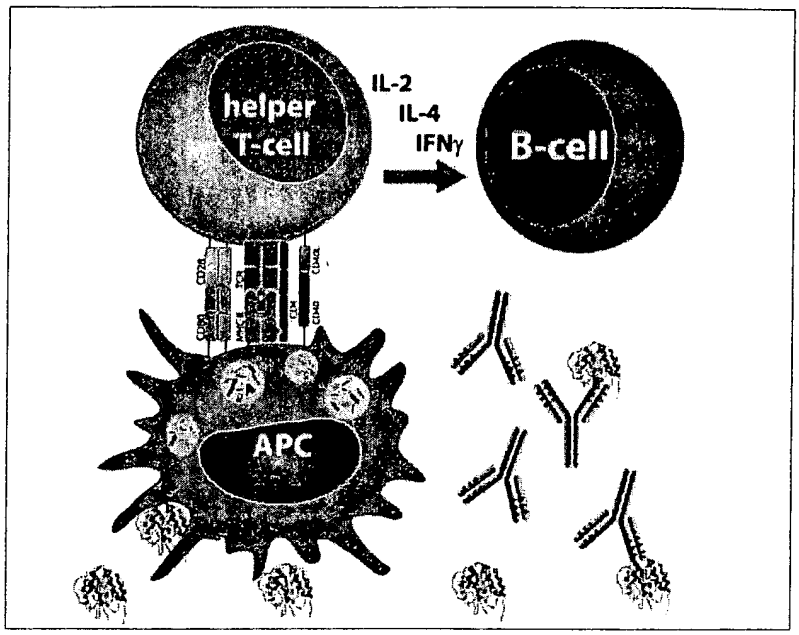

Figure 1. Schematic of T-cell activation.

B cells producing high-affinity antibodies to a therapeutic protein can only do so if stimulated by cytokines from a helper T-cell. The helper T-cell engages with an antigen presenting cell (APC) via the T-cell receptor (TCR) peptide MHC class II complex. The peptide (red) originates from the proteolytic processing of the therapeutic protein within the APC. Other co-receptors such as CD4, CD40/CD40 ligand, CD28/CD80 must also be engaged for full T-cell activation. Note that B cells also express MHC class II and act as APCs in the peripheral circulation. These can become directly activated by the T-cell.

Given that T-cell epitopes are critical risk factors to an immunogenic response, their detection in natural and engineered sequences is an important first step in helping to define the magnitude of the risk.

This then allows the opportunity to use engineering to deplete the protein of those epitopes, although this is not to ignore that other factors play a significant part in a clinical manifestation of an immune response.

Epitope prevention

A number of companies are focusing on gene shuffling or in vitro evolution techniques to produce proteins optimized for enhanced clinical benefit. Players in this field include Alligator Bioscience (Lund, Sweden), Maxygen (Redwood City, CA, USA), Diversa Inc (San Diego, CA, USA), Applied Molecular Evolution (San Diego, CA, USA, now a subsidiary of Eli Lilly and Co) and Nautilus Biotech (Evry, France), to name but a few. While some of these discovery platforms are utilizing new proteins from nature, others are engineering human proteins with enhanced properties.

These new molecules as they advance towards clinical reality will have the potential for significant immunogenicity risk in many cases. This will stem not least from their status as overtly 'foreign' proteins - for which there can be no expectation of pre-existing immunological tolerance - but also through the very engineering which has conferred the new properties. In some cases, new T-cell epitopes may be introduced either by amino acid substitution or juxtaposition of sequence modules creating junctional epitopes.

Immune risk limitation

Biovation has developed an approach, DeImmunisation, which involves the

identification and removal of T-cell epitopes as a means for limiting the risk of immunogenicity in candidate therapeutic proteins (Figure 2).

Ex vivo human T-cell proliferation assays are used to identify peptides that bind to MHC class II and stimulate the proliferation of T-cells. Peripheral blood mononuclear cells (PBMCs) isolated from the blood of healthy donors are used as a source of naive T-cells and APCs. The PBMCs are systematically allotyped and stored. The bank is continually replenished with new donor samples ensuring that multiple donors can be selected for each assay to obtain optimal coverage of MHC II allotypes within the population. Normally, overlapping peptides from the protein sequence are tested and T-cell proliferation is measured using [^{3}H]thymidine uptake. In some instances, we measure cytokine production (eg interferon gamma) as an indicator of T-helper cell activation. The result is a T-cell epitope 'map' indicating the location of T-cell epitopes within the protein.

The basic T-cell assay can be adapted into a variety of formats for particular tasks. At Biovation, time-course T-cell assays are used to study the kinetics of the T-cell response. This is useful for whole proteins or weakly immunogenic proteins. Similarly, immunological recall assays may be employed for identifying T-cell epitopes in weakly immunogenic proteins and may be configured to test peptides against patient samples in subjects known to have an immune response to a given protein drug.

Once a T-cell epitope is identified, it can usually be removed by amino acid substitution. An in silico technique termed Peptide Threading has been developed to simulate peptide-MHC II binding as a guide to selecting substitutions able to achieve epitope disruption. The inherent flexibility in choosing which substitutions to make is beneficial as it enables testing of multiple variants and selection of those that have retained functional activity of the protein. Once an epitope has been removed, the engineered peptide, and ultimately the whole protein, is tested in the T-cell assay to confirm that it is no longer capable of stimulating T-cells.

The success of the DeImmunisation approach is now being realized, with two antibodies engineered in this way so far undergoing clinical trial and each with no evidence of immunogenicity in any of the treated patients.

Epitope depletion is feasible

A recent project at Biovation has focused on the immunogenicity of interferon α (IFNα). This cytokine, administered with ribavirin, is the standard therapy for chronic hepatitis C virus infection. However, the frequent dosing required risks the development of anti-IFNα neutralizing antibodies, which correlates with reduced efficacy. Three identified immunogenic regions within IFNα have been used to produce an active variant containing mutations within these regions. Peptides spanning the modified regions were tested in T-cell assays and found to be less immunogenic than the native sequence in both PBMCs from multiple naive donors and hepatitis C virus-infected patients previously treated with IFNα.

This project and others illustrate that it is feasible to deplete proteins of T-cell epitopes and Biovation is currently engaged in further validating our findings in the run up to initiating clinical development.

Other examples

A number of other companies are focused on using immunological principles in their research and discovery phase. Thus, Genencor (Palo Alto, CA, USA), uses its Immune assay to address immunogenicity, and like Biovation, has mapped T-cell epitopes in a number of human proteins. TolerRx Inc (Cambridge, MA, USA), is using its TolerMab technology to produce therapeutic proteins with the ability to induce tolerance to themselves in the host immune system, a process likened to a molecule acting as a double agent (a tolerogen and a therapy). This attractive technology has yet to be clinically validated.

While these newer approaches await complete clinical endorsement, PEGylation offers a clinically validated option used by innovators for optimizing pharmacokinetics, increasing bioavailability and also decreasing immunogenicity. PEGylation has been likened to a putting a molecule in disguise and involves the covalent attachment of polyethylene glycol (PEG) to proteins. In aqueous solution, PEG becomes heavily hydrated and moves rapidly. It is this action which sweeps out large volumes to prevent the approach of other molecules, including immune cells. This has been suggested to decrease immunogenicity due to masking the interaction between antibodies and their epitopes on the protein.

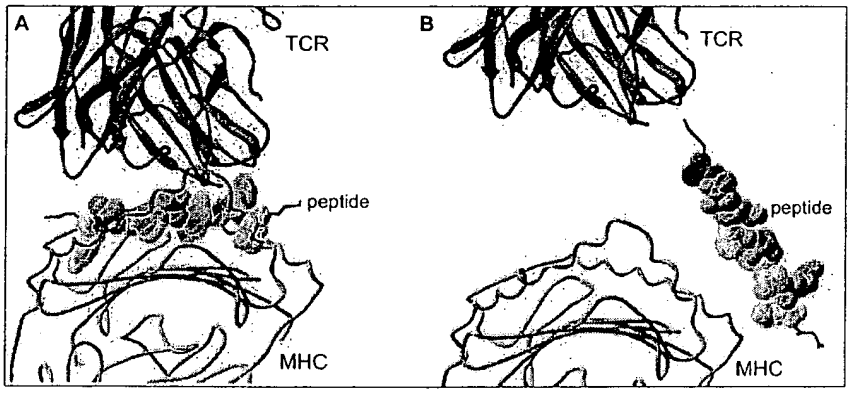

Figure 2. Elimination of a T-cell epitope by amino acid substitution.

A. Model of T-cell receptor (TCR) engaged with a peptide : MHC class II complex. In this instance the peptide acts as a T-cell epitope. B. The peptide epitope contains a substitution and is no longer able to engage the MHC and thereby function as a T-cell epitope.

PEGylation can do nothing directly about the presence of T-cell epitopes in the protein, but clearly it could be expected to have some influence over access to the APC and antigen processing.

A number of PEGylated proteins have achieved clinical registration, but PEGylation per se is no barrier to severe immune response in vivo as exemplified by the discontinuation of trials featuring PEG-thrombopoietin. Another notable PEGylated example is IFNα, which shows greatly enhanced pharmacokinetics, but there is no direct evidence to show that immunogenicity associated with the molecule has been reduced.

A critical component

As stated, many protein drugs arouse some level of antibody response in vivo. In order to assess immunogenicity, regulators are asserting that good methods for detection, quantitation and characterization of antibody responses are required during product development. Recently the working group of the Ligand Binding Assay Bioanalytical Focus Group (LBABFG) has provided recommendations on the design and optimization of immunoassays used for immunogenicity assessment of biotechnology products.

To date, the regulators have not codified specific rules for dealing with immunogenicity issues, although through recent commentaries and meetings it has been made clear that immunogenicity risk management is seen as a critical component of the development path. Immunogenicity is therefore an issue that cannot be ignored by drug developers including those seeking to bring on generic biologics for which the regulatory framework has yet to be fully constructed.

Immunogenicity risk analysis involves consideration of many different factors, including the presence or absence of an endogenous version of the product, unique or redundant biological activity, whether the product is administered as a sole or combined therapy, whether the disease is life-threatening or non life-threatening, whether the disease is chronic or end-stage, and whether or not the protein is administered as a replacement therapy, for example, as a treatment for a genetic disease.

The focus for preclinical immunogenicity testing is to understand how an immune response may affect the outcome of toxicology studies. It is important to determine when antibodies appear and what consequences these may have on therapeutic activity. Detecting product neutralization is essential as neutralizing antibodies can be expected to inhibit product toxicity in vivo. Binding antibodies, ie those which bind the product without diminishing its biological activity, may or may not mask toxicity and also need evaluation.

In practical terms, for a high-risk product undergoing a dose-escalation study, it may be necessary to monitor subjects for immune related reactions in real-time. The rationale is that in the absence of data linking dose to immunogenicity rates, it is safest to assess the immune responses of subjects to high-risk products before moving to higher doses.

At present, clinical immunogenicity data is not a general requirement for post-approval manufacturing changes if risk factors have been assessed and controlled. In assessing this risk, transgenic animal models have been used to compare immunogenicity rates between different product formulations including levels of aggregates and other factors. As animal models are in general not clinically validated, rigorous ex vivo T-cell assays could also have a role to play in this regard.

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FURTHER READING

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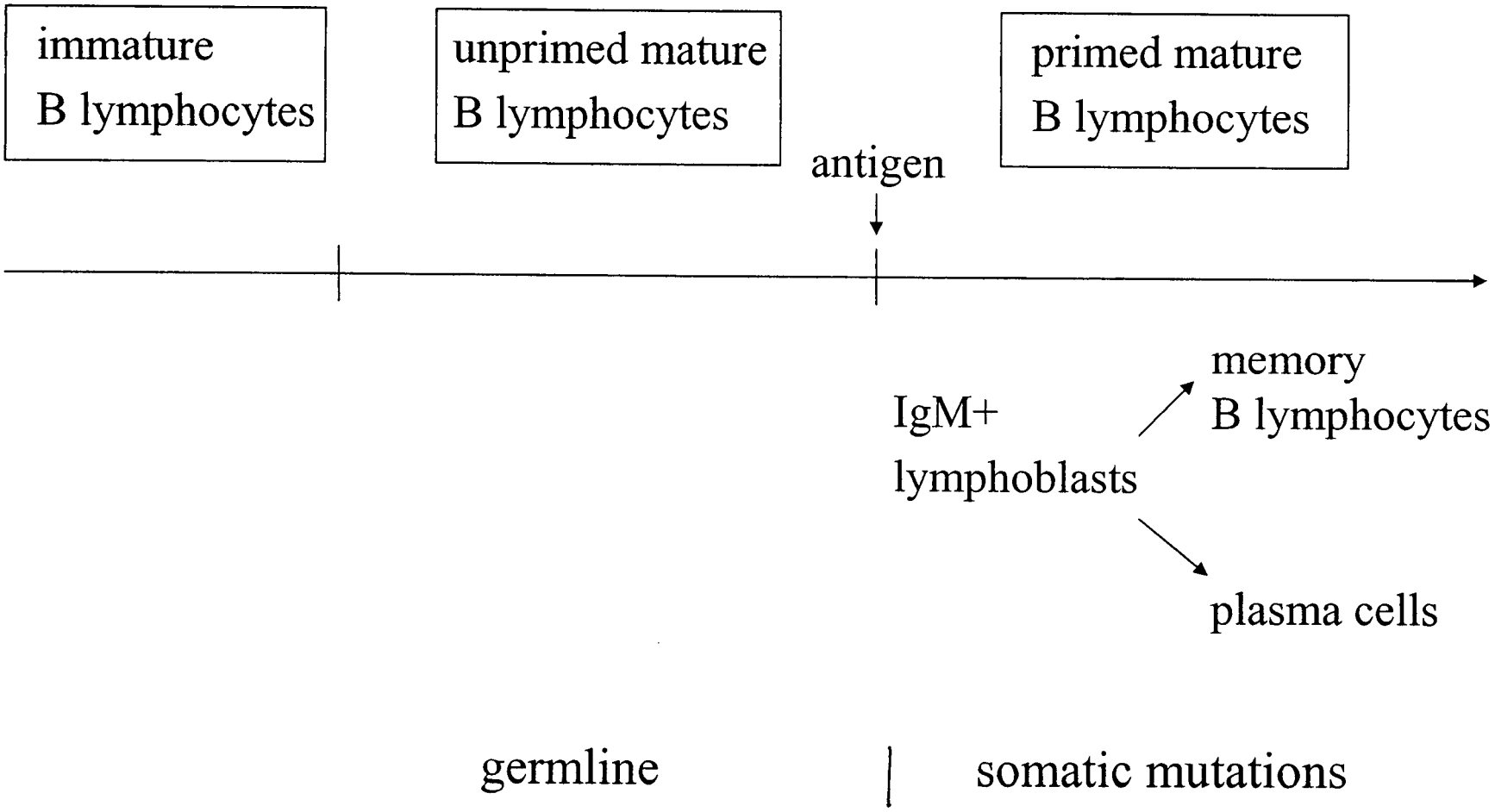
B lymphocyte maturation



Annex 1

The human VH-region D4.5 (Fig. 7) of the present invention exhibits absolute nucleotide sequence identity with the corresponding germline VH-segment DP-49 (Tomlinson, *J. Mol. Biol.* 227, 776-798 (1992)); the sequence sections to be compared (from the end of the 5`-PCR primer to the end of the genomic VH-segment) are marked with red parentheses on two separate sheets attached to Annex 1.

The absence of any nucleotide differences to the corresponding germline sequence proves derivation of D4.5 from the Ig-repertoire of mature unprimed human B-cells.

Fig. 7: D4.5:

File Name : D4.5-k8-VH

```
5'  GAG GTG CAG CTG CTC GAG TCT GGC GGA GGC GTG GTC CAG CCT GGG AGG TCC CTG
    E  V  Q  L  L  E  S  G  G  G  V  V  Q  P  G  R  S  L

    AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTC AGT AGC TAT GGC ATG CAC TGG
    R  L  S  C  A  A  S  G  F  T  F  S  S  Y  G  M  H  W

    GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG GCA GTT ATA TCA TAT GAT
    V  R  Q  A  P  G  K  G  L  E  W  V  A  V  I  S  Y  D

    GGA AGT AAT AAA TAC TAT GCA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA
    G  S  N  K  Y  Y  A  D  S  V  K  G  R  F  T  I  S  R

    GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCT GAG GAC
    D  N  S  K  N  T  L  Y  L  Q  M  N  S  L  R  A  E  D

    ACG GCT GTG TAT TAC TGT GCG AAA GAT ATG GGG TGG GGC AGT GGC TGG AGA CCC
    T  A  V  Y  Y  C  A  K  D  M  G  W  G  S  G  W  R  P

    TAC TAC TAC TAC GGT ATG GAC GTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC
    Y  Y  Y  Y  G  M  D  V  W  G  Q  G  T  T  V  T  V  S

    TCA GCA CCC ACC AAG GCT CCG GAT GTG TTC CCT CTA 3'
    S  A  P  T  K  A  P  D  V  F  P  L
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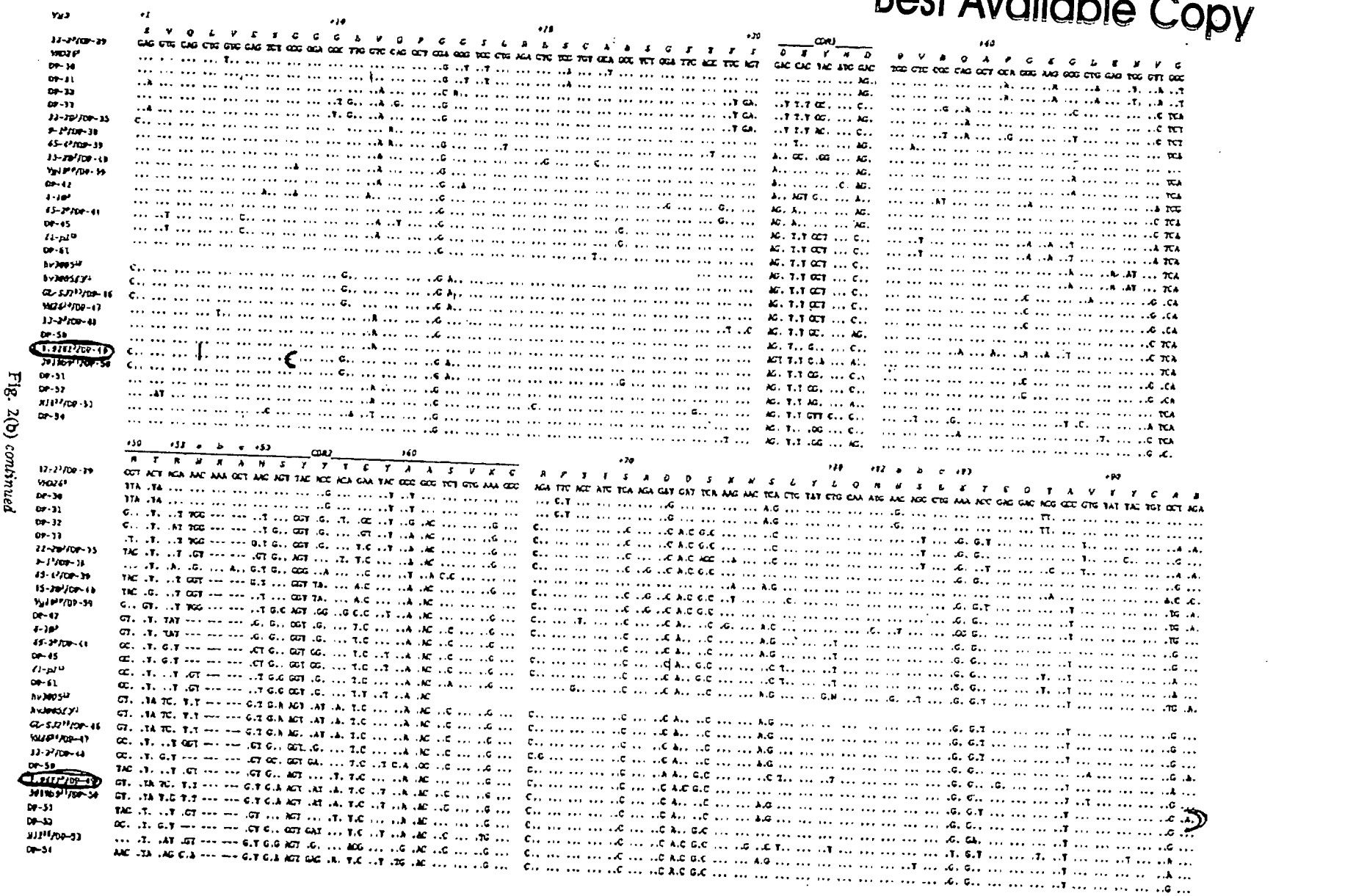

Fig. 2(b) *continued*

Annex 2

The human VH-region D7.2 (Fig.8) of the present invention is the result of a PCR-crossover between the two different germline VH-segments DP-33 and DP-49 (Tomlinson, *J.Mol.Biol.* 227, 776-798 (1992)). As marked with red parentheses on four seperate sheets attached to Annex 2, D7.2 exhibits absolute nucleotide sequence identity between the end of the 5`-PCR primer and the PCR-crossover with the corresponding sequence section of DP-33 as well as between the PCR-crossover and the end of the genomic VH-segment with the corresponding sequence section of DP-49; the PCR-crossover is marked with a blue arrow.

Both parts of D7.2 are free of nucleodide differences to the corresponding germline sequences and therefore derived from the repertoire of mature unprimed human B-cells, respectively.

Fig. 8:

File Name : D7.2-k8-VH

```
                9                  18                  27                  36                  45                  54
5' GAG GTG CAG CTG CTC GAG TCT GGG GGA GTC GTG GTA CAG CCT GGG GGG TCC CTG
   E   V   Q   L   L   E   S   G   G   V   V   V   Q   P   G   G   S   L

               63                  72                  81                  90                  99                 108
   AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTT GAT GAT TAT GCC ATG CAC TGG
   R   L   S   C   A   A   S   G   F   T   F   D   D   Y   A   M   H   W

              117                 126                 135                 144                 153                 162
   GTC CGC CAG GCT CCA GGC AAG GGG CTG GAG TGG GTG GCA GTT ATA TCA TAT GAT
   V   R   Q   A   P   G   K   G   L   E   W   V   A   V   I   S   Y   D

              171                 180                 189                 198                 207                 216
   GGA AGT AAT AAA TAC TAT GCA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA
   G   S   N   K   Y   Y   A   D   S   V   K   G   R   F   T   I   S   R

              225                 234                 243                 252                 261                 270
   GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG AAC AGC CTG AGA GCT GAG GAC
   D   N   S   K   N   T   L   Y   L   Q   M   N   S   L   R   A   E   D

              279                 288                 297                 306                 315                 324
   ACG GCT GTG TAT TAC TGT GCG AAA AAG GAA GGC TAC TGG GGC CAG GGA ACC CTG
   T   A   V   Y   Y   C   A   K   K   E   G   Y   W   G   Q   G   T   L

              333                 342                 351                 360                 369
   GTC ACC GTC TCC TCA GCA CCC ACC AAG GCT CCG GAT GTG TTC CCT CTA 3'
   V   T   V   S   S   A   P   T   K   A   P   D   V   F   P   L
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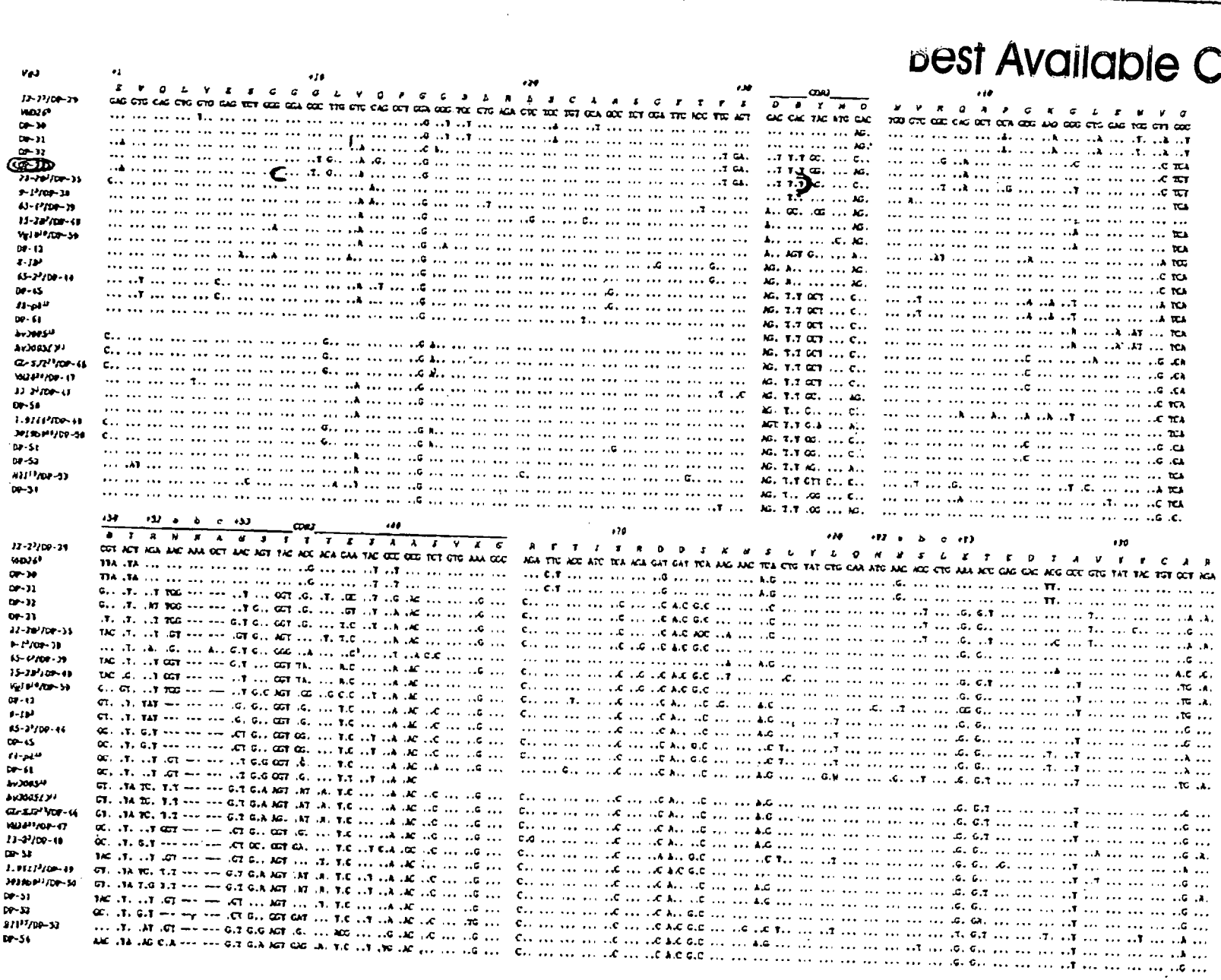
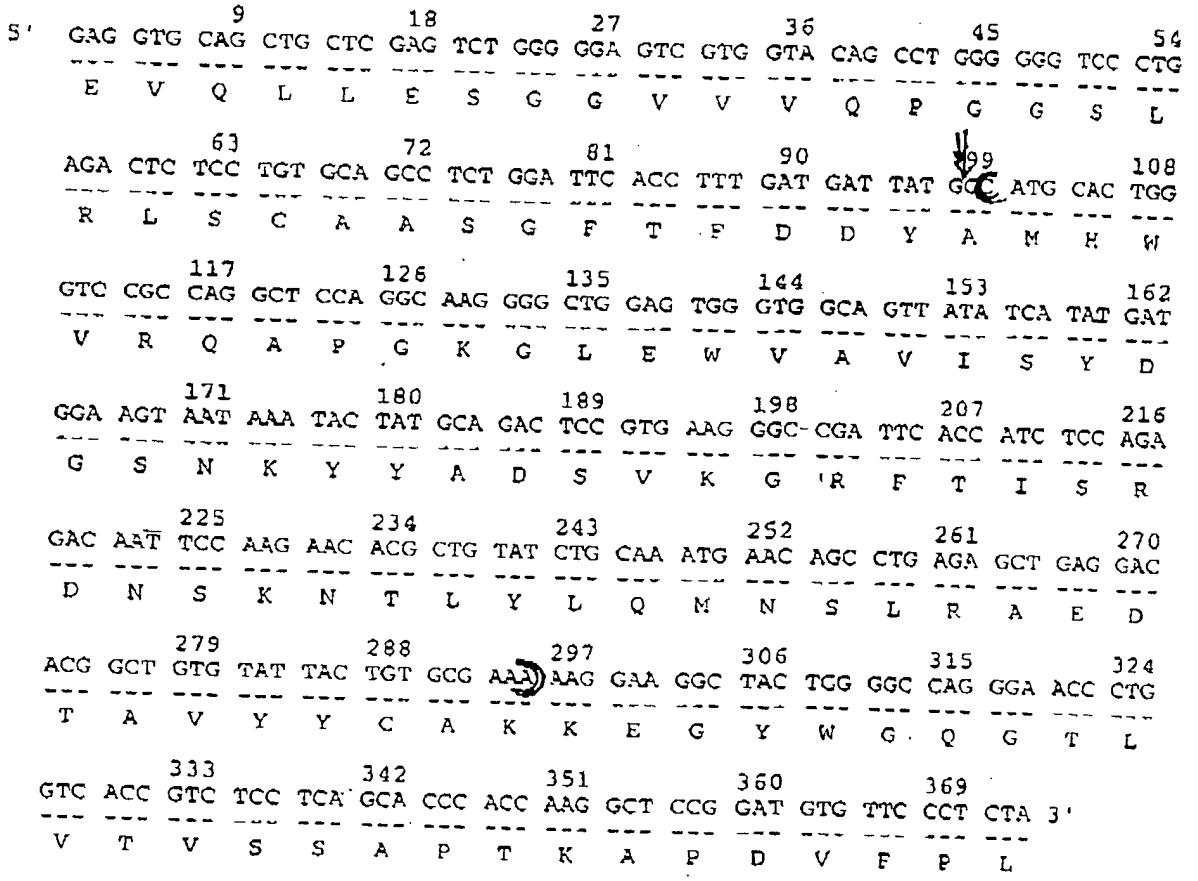


Fig. 2(b) *continued*

Fig. 8:

File Name : D7.2-k8-VH



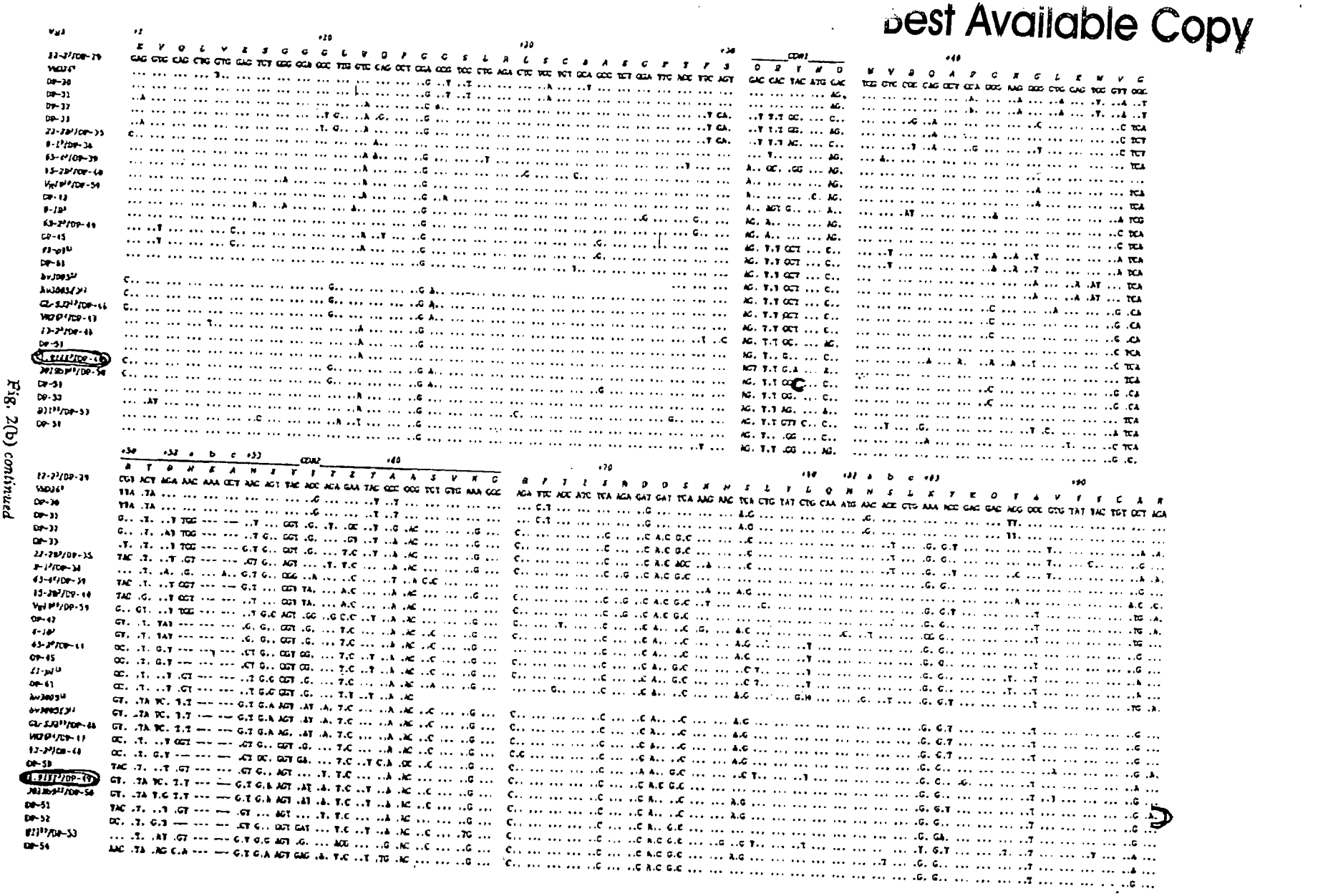


Fig. 2(b) continued

Annex 3

Absence of nucleotide exchanges from the VH-region of human antibody αThy-29 compared to the corresponding germline VH-segment DP-14 is claimed in document D3, Table II at p.76. However, comparison of DP-14 (Tomlinson, *J. Mol. Biol.* 227, 776-798 (1992)) with the VH-sequence of αThy-29 (Griffiths, *EMBO J.* 12, 725-734 (1993), Genebank Accession Number Z18832) reveals two nucleotide differences to the germline sequence. The sequence sections to be compared (from the end of the 5`-PCR primer to the end of the genomic VH-segment; in red parentheses) as well as the nucleotide exchanges (green circles) are marked on two separate sheets attached to Annex 3.

The presence of nucleotide exchanges compared to the corresponding germline sequence excludes derivation of αThy-29 from the Ig-repertoire of mature unprimed human B-cells.

NCBI Entrez Nucleotide QUERY BLAST Entrez ?

THY VH 29

Other Formats: FASTA Graphic

Links: MEDLINE Protein Related Sequences

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LOCUS       HSIGHVBAF     367 bp    DNA                 PRI       08-AUG-1995
DEFINITION  H.sapiens rearranged Ig H-chain V-domain cDNA.
ACCESSION   Z18832
NID         g33115
VERSION     Z18832.1  GI:33115
KEYWORDS    Ig heavy chain variable region; immunoglobulin.
SOURCE      human.
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 367)
  AUTHORS   Griffiths,A.D.
  TITLE     Direct Submission
  JOURNAL   Submitted (25-NOV-1992) Griffiths A.D., MRC Centre for Protein
            Engineering, Hills Road, Cambridge, CB2 2QH, U.K
REFERENCE   2  (bases 1 to 367)
  AUTHORS   Griffiths,A.D., Malmqvist,M., Marks,J.D., Bye,J.M., Embleton,M.J.,
            McCafferty,J., Baier,M., Holliger,K.P., Gorick,B.D.,
            Hughes-Jones,N.C., Hoogenboom,H.R. and Winter,G.
  TITLE     Human anti-self antibodies with high specificity from phage display
            libraries
  JOURNAL   EMBO J. 12 (2), 725-734 (1993)
  MEDLINE   93178448
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                     /macronuclear
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                     /sex="Male"
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                     /cell_type="peripheral lymphocyte"
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                     /gene="IgH"
     CDS             <1..>367
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                     IDDFWSGYNFDYWGQGTLVT"
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       61 tcctgcaagg cttctggtta caccttacc agctatggta tcagctgggt gcgacaggcc
      121 cctggacaag ggcttgagtg gatgggatgg atcagcgctt acaatggtaa cacaaactat
      181 gcacagaagc tccagggcag agtcaccatg accacagaca catccacgag cacagcctac
      241 atggagctga ggagcctgag atctgacgac acggccgtgt attactgtgc gcgagatacg
      301 gggaggatcg acgatttttg gagtggttac aactttgact actggggcca gggaaccctg
      361 gtcaccg
//
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two green circles

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Fig. 2(b)

Annex 4

Absence of nucleotide exchanges from the VH-region of human antibody αTNF-E7 compared to the corresponding germline VH-segment DP-10 is claimed in document D3, Table II at p.76. However, comparison of DP-10 (Tomlinson, *J. Mol. Biol.* 227, 776-798 (1992)) with the VH-sequence of αTNF-E7 (Griffiths, *EMBO J.* 12, 725-734 (1993), Genebank Accession Number Z18841) reveals one nucleotide difference to the germline sequence. The sequence sections to be compared (from the end of the 5`-PCR primer to the end of the genomic VH-segment; in red parentheses) as well as the nucleotide exchanges (green circles) are marked on two separate sheets attached to Annex 4.

The presence of a nucleotide exchange compared to the corresponding germline sequence excludes derivation of αTNF-E7 from the Ig-repertoire of mature unprimed human B-cells.

NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA Graphic TNF VH E7

Links: MEDLINE Protein Related Sequences

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DEFINITION  H.sapiens rearranged Ig H-chain V-domain cDNA.
ACCESSION   Z18841
NID         g33119
VERSION     Z18841.1  GI:33119
KEYWORDS    Ig heavy chain variable region; immunoglobulin.
SOURCE      human.
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 348)
  AUTHORS   Griffiths,A.D.
  TITLE     Direct Submission
  JOURNAL   Submitted (25-NOV-1992) Griffiths A.D., MRC Centre for Protein
            Engineering, Hills Road, Cambridge, CB2 2QH, U.K
REFERENCE   2  (bases 1 to 348)
  AUTHORS   Griffiths,A.D., Malmqvist,M., Marks,J.D., Bye,J.M., Embleton,M.J.,
            McCafferty,J., Baier,M., Holliger,K.P., Gorick,B.D.,
            Hughes-Jones,N.C., Hoogenboom,H.R. and Winter,G.
  TITLE     Human anti-self antibodies with high specificity from phage display
            libraries
  JOURNAL   EMBO J. 12 (2), 725-734 (1993)
  MEDLINE   93178448
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                     LEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARGPLR
                     GYDYYYYYMDVWGK"
BASE COUNT        79 a     86 c    115 g     68 t
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       61 tcctgcaagg cttctggagg caccttcagc agctatgcta tcagctgggt gcgacaggcc
      121 cctggacaag ggcttgagtg gatgggaggg atcatccctа tctttggtac agcaaactac
      181 gcacagaagt tccagggcag agtcacgatt accgcggacg aatccacgag cacggcctac
      241 atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc gagagggccg
      301 cttcgtgggt atgactacta ctactactac atggacgtct ggggcaaa
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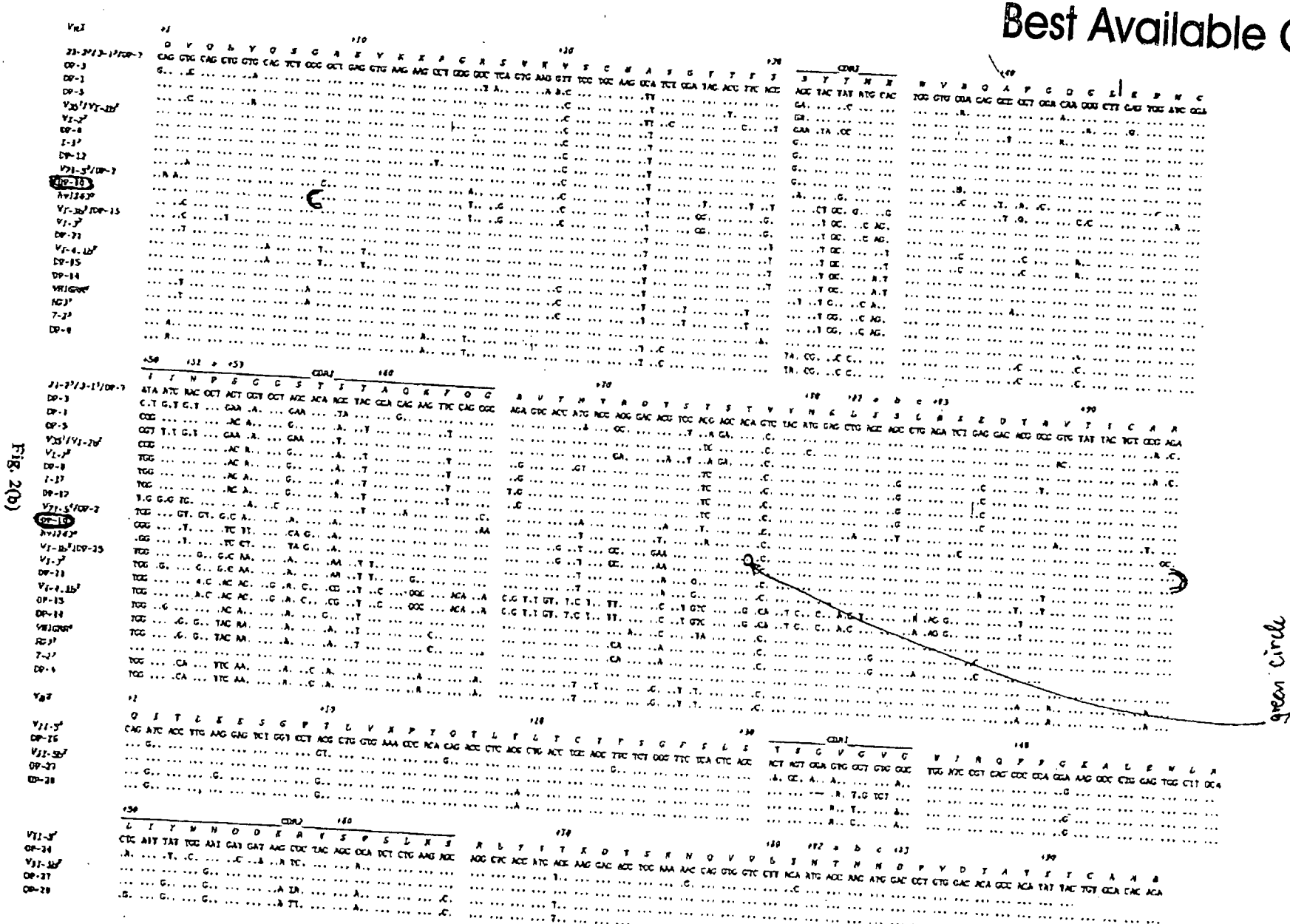

Fig. 2(b)

green circle

Annex 5

The human VH-region αMUC1-1 of document D3 is the result of a PCR-crossover between the two different germline VH-segments VI-2 and DP-25 (Tomlinson, *J. Mol. Biol.* 227, 776-798 (1992)). In contrast to the information given in D3, Table II at p.76, three instead of two nucleotide exchanges compared to the corresponding germline sequences are found in VH of αMUC1-1 (Griffiths, *EMBO J.* 12, 725-734 (1993), Genebank Accession Number Z18846). The sequence sections to be compared (from the end of the 5`-PCR primer to the PCR-crossover; in red parentheses) as well as the nucleotide exchanges (green circles) are marked on two separate sheets attached to Annex 5; the PCR-crossover is marked with a blue arrow.

The presence of nucleotide exchanges compared to the corresponding germline sequences excludes derivation of αMUC1-1 from the Ig-repertoire of mature unprimed human B-cells.

NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA Graphic

Links: MEDLINE Protein Related Sequences

Muc I VH I

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LOCUS       HSIGHVBAL     354 bp    DNA              PRI       08-AUG-1995
DEFINITION  H.sapiens rearranged Ig H-chain V-domain cDNA.
ACCESSION   Z18846
NID         g33121
VERSION     Z18846.1  GI:33121
KEYWORDS    Ig heavy chain variable region; immunoglobulin.
SOURCE      human.
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 354)
  AUTHORS   Griffiths,A.D.
  TITLE     Direct Submission
  JOURNAL   Submitted (25-NOV-1992) Griffiths A.D., MRC Centre for Protein
            Engineering, Hills Road, Cambridge, CB2 2QH, U.K
REFERENCE   2  (bases 1 to 354)
  AUTHORS   Griffiths,A.D., Malmqvist,M., Marks,J.D., Bye,J.M., Embleton,M.J.,
            McCafferty,J., Baier,M., Holliger,K.P., Gorick,B.D.,
            Hughes-Jones,N.C., Hoogenboom,H.R. and Winter,G.
  TITLE     Human anti-self antibodies with high specificity from phage display
            libraries
  JOURNAL   EMBO J. 12 (2), 725-734 (1993)
  MEDLINE   93178448
FEATURES             Location/Qualifiers
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Fig. 2(b)

Annex 6

Annex 6